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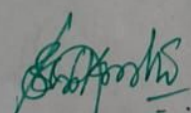
Certificate of Completion

This is to certify that **Mr. P. S. Karthik**, has successfully completed his project work on "**Anticancer activity of *Blumea mollis* and *Hyptis suaveoleus* plant extracts on Human Skin and Lung Cancer cell lines** " from 10th Jun' 2011 to 10th Sep' 2011.

Date Issued: 10/09/2011

At: Visakhapatnam




Sashikanth. C

Director

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To whomsoever it may Concern

It gratifies me to give this letter of recommendation for Mr. Karthik. I know him as a project student under my supervision at Translational Research Institute of Molecular Sciences from June to September 2011 for project titled "Anticancer Activity of Blumea Mollis and Hypis Seaveolus on skin and lung cancer cell line". At our very first meeting I briefed the broad summary of the project by which he might work on demonstrated the ability to effort independently with great creativity and enthusiasm. He has impressed me with his clarity of thought through good communication skills. His proficiency in articulating his ideas is clearly perceptible.

The other student working with him commented favorably. He got along well with everyone, pulled his own load along with colleague during dissertation, and had the skill to adjust with other team members. From the very first day he continued to read books, literatures & independently generated interesting new hypothesis. He is awarded as second author in the publication in June 2012 titled "Evaluation of Antioxidant and Anticancer Properties of Methanolic Extracts of Abutilon indicum and Blumea mollis" at Journal of Pharmacy Research

In summary, He is highly motivated, intelligence, perseverance and committed towards his work with sincere and hardworking nature. Which I obviously believe as a distinctive construction for his research carrier. I will give him my highest recommendation. I strongly consider that he will do high quality research and it is a necessary step towards his career goal. I hope that you will find him as deserving and creditable candidate for your renowned organization / institution.



Sincerely,

Sashikanth. C
Director

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Evaluation of Antioxidant and Anticancer Properties of Methanolic Extracts of *Abutilon indicum* and *Blumea mollis*

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ABSTRACT

Successful plant remedies and their preparations as medicinal treatments have been used for thousands of years in indigenous cultures around the world. Many of today's valuable drugs such as aspirin, digitoxin, morphine and quinine are resulted from traditionally used plants after scientific evaluation. In the present study medicinal plants namely *Abutilon indicum* and *Blumea mollis* were chosen to screen for potential anti-oxidant properties and cytotoxic activity. The concentration of phenol was determined using Folin-Ciocalteu assay (26) with minor modifications. The extract was also screened to assess the antioxidant activity using FRAP, 1, 1-Diphenyl-2-picrylhydrazyl [DPPH] radical scavenging activity and Nitric Oxide radical inhibition estimated by the use of Griess Illosvoy reaction (6) with slight modification. These extracts show anti-oxidant properties as well as inhibitory effect on cancer cells with the increased concentration and duration. When used in higher concentrations, these extracts show good inhibition rates which might prove to be effective for the prevention of cancer.

Keywords: *Abutilon indicum*; *Blumea mollis*; Antioxidant properties; Cytotoxic activity; DPPH; MEM

INTRODUCTION

Reactive oxygen species (ROS), such as superoxide anions, hydrogen peroxide, and hydroxyl, nitric oxide and peroxynitrite radicals, play an important role in oxidative stress related to the pathogenesis of various important diseases (19, 20). Williams (22) reported that the body handles this cell damage process by producing a number of antioxidant enzymes which can be found both in the human body and in plants (23). Thus, antioxidants protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxy radicals, hydroxyl radicals and peroxynitrite.

Cancer kills annually about 3500 per million populations around the world. A large number of chemo preventive agents are used to cure various cancers, but they produce side effects that prevent their excess usage. Cancer has become a main interest of natural products chemistry over the years due to its widespread diagnosis. Natural products research has introduced many revolutionary compounds that have been used as ingestible remedies for a variety of health concerns and problems throughout the years. The importance of medicinal plants in the management of human ailments cannot be over emphasized. Natural products chemistry has brought forth compounds that have gone on to eventually lead to advanced cancer treatments, pain relievers, dietary. Since present medication and treatments producing side effects to the patients, there is a search for a traditional type of medication and can be proved as taxonomical markers (3, 14).

The medicinal plants harbor an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Natural products from medicinal plants are known to be chemically balanced, effective and least injurious with none or much reduced side effects as compared to syn-

thetic medicines. Several bioassays are available to screen crude plant extracts for their potential for a specific biological activity. Therefore the main aim of this study is to determine the antioxidant and anticancer properties of methanolic extracts of *Abutilon indicum* and *Blumea mollis*.

MATERIALS AND METHODS

Preparation, separation and concentration of plant extract:

Medicinal plants namely *Abutilon indicum* and *Blumea mollis* were authenticated. The air-dried and powdered whole plant materials were extracted thrice with methanol. The methanolic extract was concentrated under reduced pressure using the rotary evaporator at a temperature of 40°C. Concentrated methanolic extract were lyophilized to dryness using freeze drier. The powdered samples were stored at 4°C and used within seven weeks after production.

Antioxidant Studies:

Total Phenolic content determination assay:

Total natural phenolic of plant extracts was determined using Folin-Ciocalteu assay (26) with minor modifications. Test tube containing either 500ul of either standard solutions of gallic acid (50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125ug/ml) or crude extracts (diluted 400-fold with distilled deionized water) was prepared, 500ul of 10% Folin-Ciocalteu's phenol reagent (in DDW) was added into each test tube and mixed. After 20min, 350ul of 1M Na₂CO₃ solution was added into the mixture. After incubation for 20min at room temperature, the absorbance was determined at 750nm against the parallelly prepared blank (500ul of DDW +500ul of 10% FC reagent + 350ul of 1M Na₂CO₃ solution).

Determination of DPPH radical scavenging activity:

The free radical scavenging activity of the extracts was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH (2, 2-diphenyl-1-picrylhydrazyl). 0.1 mM solution of DPPH in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution

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in water at different concentrations (250-2500 µg/ml). Thirty minutes later, the absorbance was measured at 517 nm. Ascorbic acid was used as a standard. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH Scavenged (\%)} = (A_{\text{control}} - A_{\text{test}} / A_{\text{control}}) \times 100$$

Where **A** control is the absorbance of the control reaction and **A** test/std is the absorbance in the presence of the extracts. The antioxidant activity of the extracts was expressed as IC₅₀. The IC₅₀ value was defined as the concentration in µg/ml of extracts that inhibits the formation of DPPH radicals by 50%.

Reducing power assay:

The reducing power of methanolic extracts was determined according to the method of Oyaizu (1986). Different concentrations of pod extract (10– 100 µg/ml) in 1ml of methanol were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferrocyanide (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl₃ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm and compared with standard. Increased absorbance of the reaction mixture indicated increased reducing power.

Determination of nitric oxide radical scavenging activity:

Nitric Oxide radical inhibition estimated by the use of Griess Illosvoy reaction (6). Nitric oxide was generated from sodium nitroprusside and measured by the Griess reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitric ions that can be estimated by use of Griess reagent. Scavenger of nitric oxide competes with oxygen leading to reduced production of nitric oxide. Sodium nitroprusside (5 mM) in phosphate-buffered saline (PBS) was mixed with 3.0 ml of different concentrations (10-100 µg /ml) of the drugs dissolved in the suitable solvent systems and incubated at 25°C for 150 min. The samples from the above were reacted with Griess reagent (1% sulphanilamide, 2% H₃PO₄ and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine was read at 546 nm.

Anticancer studies:

Materials:

Serum containers, 15ml centrifuge tubes (TARSONS), 50ml Falcon (TARSONS), Glycerol, Minimal essential medium (MEM), Trypsin-EDTA, T25-flasks (Corning), Tissue Culture Plates for MTT Assay (Nunc), MTT (SIGMA).

Culture Medium:

The culture medium (CM) consisted of MEM (Gibco) supplemented with 10% heat-inactivated fetal bovine serum (Gibco), 100 U/mL of penicillin and 100 mg/mL of streptomycin.

Cell lines and culture conditions:

Human cancer cell lines SK-MEL-28 (Melanoma) and NCI-H23 (Lung Adeno Carcinoma) were procured from National Centre for Cell Science, Pune. All cells were grown in Minimal essential medium (MEM,

GIBCO). Cultures were maintained at 5% CO₂ and 37°C temperature.

Cytotoxicity assay: NCI-H23 (3-5 × 10³ per well) and SK-MEL28 (8-12 × 10³ per well) cell lines were plated in 96-well plates in complete medium and treated in triplicate with different concentrations of plant extracts or 0.1% DMSO (control) for 72 hours. Cytotoxic potential of crude plant extracts on these cancer cell lines was estimated using MTT assay. The assay is based on the ability of dehydrogenases of living cells to convert 3-(4,5-dimethylthiazole-2-yl)-2,5-tetrazolium bromide (ÏÖÖ) of yellow color into insoluble purple-and-blue crystals of ÏÖÖ-formazan, which were extracted by isopropanol and determined on reader (Microplate Reader 2010, Anthos Biochrom) at Ë=570 nm. Intensity of conversion ÏÖÖ>ÏÖÖ-formazan reflects the total level of dehydrogenase activity of cells, i.e. a final effect of cytotoxicity of injected preparation (28). MTT assay is advantageous in being rapid and precise without any radio isotope. Reduction of MTT takes place when reductase enzymes are active, and therefore conversion is often used as a measure of viable or living cells.

$$\% \text{ cell survival} = 100 - \{(A_{\text{t}} - A_{\text{b}}) / (A_{\text{c}} - A_{\text{b}})\} \times 100$$

Where: **A_t** = Absorbance of test, **A_b** = Absorbance of blank, **A_c** = Absorbance of control

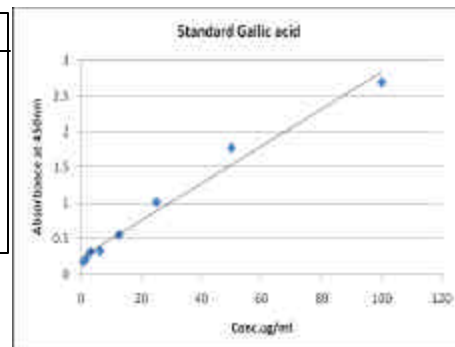
$$\% \text{ cell inhibition} = 100 - \% \text{ cell survival}$$

RESULTS AND DISCUSSION

In this study, DPPH, FRAP and Nitric oxide free radical scavenging were used to evaluate the antioxidant activity of methanolic extracts of *A. indicum* and *B. mollis*. The results show that the rate of free radical scavenging effect of *B. mollis* on DPPH was good especially that of *A. indicum* which was better than Ascorbic acid and BHT. A similar observation was made using different concentrations of the extract suggesting that the *A. indicum* and *B. mollis* possess strong antioxidant properties. The strong antioxidant activity demonstrated by the two species of *A. indicum* and *B. mollis* confirmed that they are free radical scavengers possibly as primary antioxidant that reacts with free radicals, particularly of the peroxy radicals, which are the major propagation of the autoxidation chain of fats, thereby terminating free radical chain reaction (23, 24, 25). This can be attributed to the antioxidant activity of the extract provided by the chemical constituents of the plant (26). Thus the mechanism may be derived from the action of the chemical compounds, flavonoids and phenols in the plant medicine (29). These findings therefore justify the use of these medicinal plants in the treatment of several ailments in India.

Total Phenolic content: Standard Gallic acid

Conc. In ug/ml	Abs. at 750nm
100	2.695
50	1.776
25	1.012
12.5	0.558
6.25	0.331
3.125	0.316
1.562	0.221
0.781	0.178



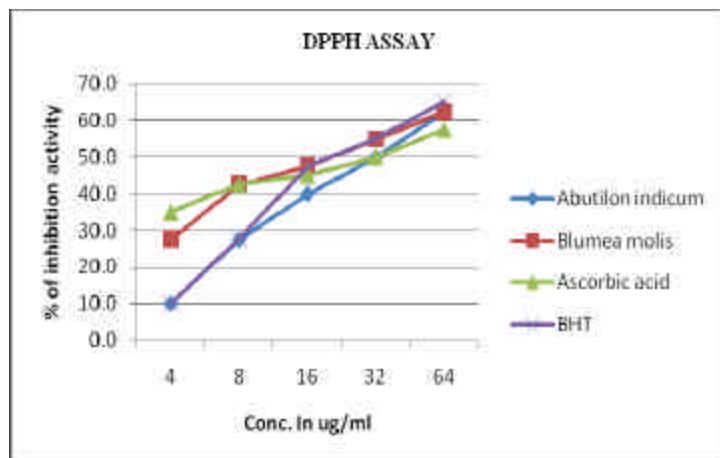
Total Phenolic content:

Abutilon indicum: 14.93µg GAE/mg

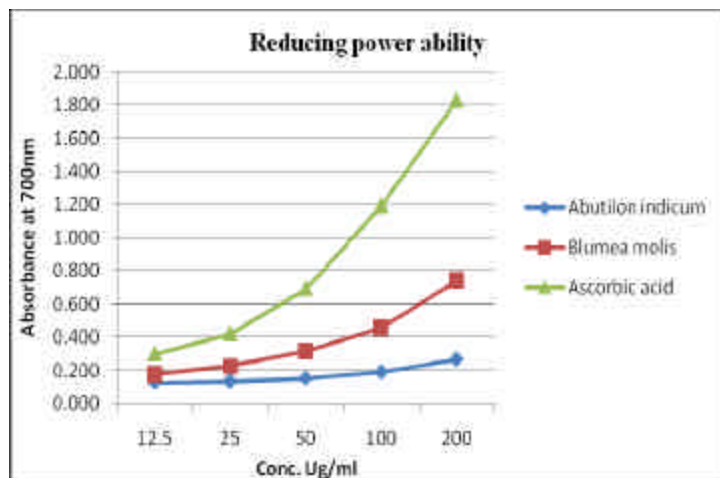
Blumea mollis: 101µg GAE/mg

DPPH Radical scavenging activity

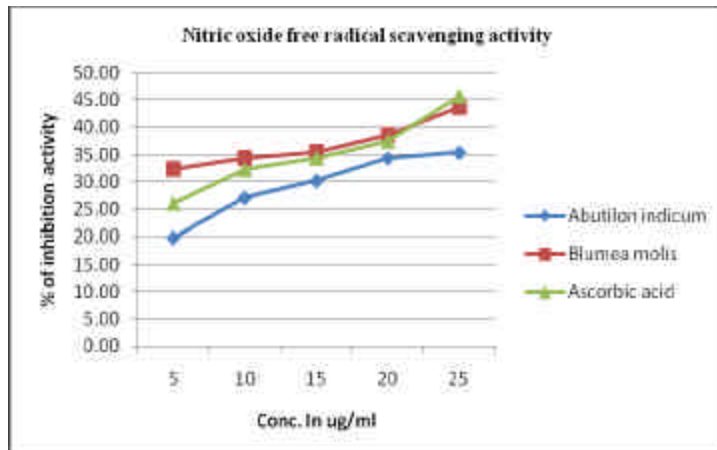
DPPH ASSAY % of Inhibition activity				
Conc. In ug/ml	Ab	Blu	Asc.	BHT
4	10.0	27.5	35	10
8	27.5	42.5	42.5	27.5
16	40	47.5	45	47.5
32	50.0	55	50.0	55
64	62.5	62.5	57.5	65

**Reducing power ability:**

Absorbance at 700nm			
Conc. In ug/ml	Ab	Blu	Asc.
12.5	0.129	0.176	0.300
25	0.134	0.224	0.422
50	0.153	0.318	0.695
100	0.191	0.456	1.194
200	0.268	0.744	1.830

**Nitric oxide free radical scavenging activity**

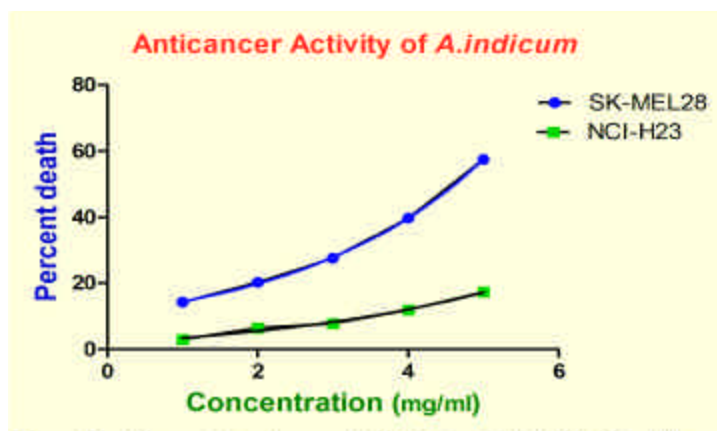
Nitric oxide free radical scavenging			
Conc. In ug/ml	Ab	Blu	Asc.
5	19.79	32.29	26.04
10	27.08	34.37	32.29
15	30.20	35.41	34.37
20	34.37	38.54	37.50
25	35.41	43.75	45.8



Methanolic extract of *A. indicum* was studied for inhibitory property on human melanoma (SK-MEL28 cell lines) and human non-small cell lung cancer (NCI-H23 cell lines) by MTT assay. *A. indicum* has shown IC₅₀ value of 4.71 mg/ml on SK-MEL28 and IC₅₀ value 15.8 mg/ml on NCI-H23 cell lines. Methanolic extract of *B. mollis* at 5mg concentration exhibited a high inhibition rate of 49.10% (IC₅₀ value 6.31 mg/ml) on SK-MEL-28 cell lines and 57.84% inhibition rate at 5mg concentration (IC₅₀ value 4.45 mg/ml) on NCI-H-23 cell lines.

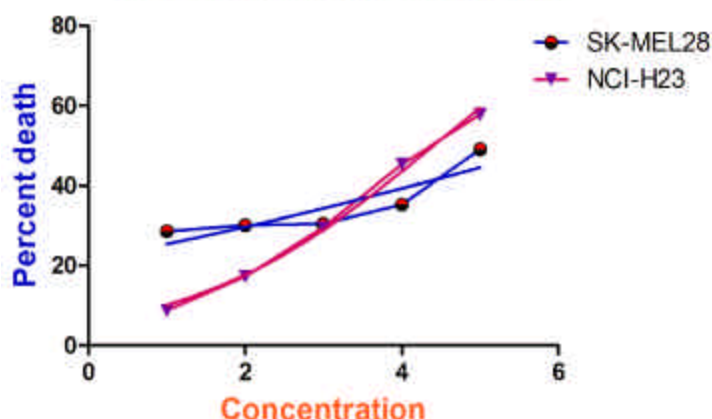
Table 1: Shows the effect of *A.indicum* on SK-MEL-28 and NCI-H23 cell lines

Concentration of <i>A.indicum</i> (mg/ml)	% of inhibition SK-MEL-28 cell line	NCI-H23 cell line	IC ₅₀ values SK-MEL-28 cell line	NCI-H23 cell line
1	14.26	2.94	4.71	15.8
2	20.21	6.52		
3	27.50	7.78		
4	39.79	9.90		
5	57.52	17.29		

**Graph 1: Effect of *A.indicum* on NCI-H23 and SK-MEL-28 cell lines****Table 2: Shows the effect of *B.mollis* on SK-MEL-28 and NCI-H23 cell lines**

Concentration of <i>B. mollis</i> (mg/ml)	% of inhibition SK-MEL-28 cell line	NCI-H23 cell line	IC ₅₀ values SK-MEL-28 cell line	NCI-H23 cell line
1	28.57	8.75	6.31	4.45
2	30.10	17.21		
3	30.44	29.55		
4	35.23	45.27		
5	49.10	57.84		

Anticancer Activity of *B. mollis*



Graph 2: Effect of *B. mollis* on SK-MEL-28 and NCI-H23 cell lines

CONCLUSION

Previous studies on *Abutilon indicum* showed this plant contains Alanto lactone, isolactone and β -sitosterol compounds which have anti-bacterial and anti-fungal properties while *Blumea mollis* with Linalool and alloxanthrene has shown to possess anti-bacterial and anti-fungal properties. Both the medicinal plants exhibited varying sensitivity on cell lines on NCI-H23 and SK-MEL-28 cell lines. So, this study shows that these plants contain anti-cancer properties and can exhibit better response when used in higher concentration.

REFERENCES

- Alejandra Rojas, L. H.-P.-M. (1992). Screening for antimicrobial activity of crude drug extracts and pure natural products from Mexican medicinal plants. *Journal of Ethnopharmacology*, 275-283.
- Carlo M. Croce, M. (2008). Oncogenes and cancer. *The New England Journal of Medicine*, 502-511.
- C.K.K.Nair, P. a. (2010). Ethnomedicinal plants to fight neoplastic diseases. *Research signpost*, 203-226
- Das Prabhat K., S. S. (2009). Phytochemical and pharmacological investigation of the protective effect of plants *hyptis suaveolens* against duodenal ulceration. *Journal of Global Pharma Technology*, 82-87.
- Jaoa Sammy N. Souza, L.L. (2005). Pyrrolizidine alkaloids from *heliotropium indicum*. *J.Braz.chem.soc*, 1410-1414.
- Garrat DC (1964). *The Quantitative analysis of drugs*. Chapman and Hall Ltd, Japan. 3: 456-458.
- J.Fidler, I. (1990). Critical Factors in the biological of human cancer metastasis: Twenty-eighth G.H.A.Clones memorial award lecture. *American Association of cancer research*, 6130-6138.
- John S.kovach, M. M. (1979). Toxicity and pharmacokinetics of a pyrrolizidine alkaloid, indicine N-oxide in humans. *American association of cancer research*, 4540-4544.
- Jyothi KS, H. P. (2011). Evaluation of three medicinally important traditional wild food plants of India. *International Journal of Green Pharmacy*, 95-99.
- K.srinivas, M. (2000). Anti inflammatory activity of *heliotropium indicum* linn and *leucas asperaspren* in albino rats. *Indian Journal of Pharmacology*, 37-38.
- Moulik.C, V.T. (2009). Phytoresources as potential therapeutic agents for cancer treatment and prevention. *Journal of Global Pharma Technology*, 4-17.
- M.A Rahman, M.M. (2011). Pharmacological and phytochemical screen activities of roots of *heliotropium indicum* linn. *Pharmacologyonline*, 185-192.
- N. peerzada. (1997). Chemical composition of the essential oil of *Hyptis suaveolens*. *Molecules*, 165-168.
- Pandey, S. a. (2009). Some anticancer medicinal plants of foreign origin. *Current sciences*, 779-783.
- Prakash S.Sukhrmani, P.S. (2011). In-vitro cytotoxicity evaluation of novel N-substituted bis-benzimidazole derivatives for anti-lung and anti-breast cancer activity. *Annals of biological research*, 51-59.
- P.N.,D.G. (2011). Antimicrobial activity of few medicinal plants. *International Research Journal of Pharmacy*, 146-152.
- R.S.Costa, S.C.B. (2011). Physical, chemical and physico-chemical control of *heliotropium indicum* linn, boraginaceae, powder and tincture. *International Journal of Pharmaceutical Science and Research*, 2211-2216.
- Venkatesalu, A. S.-K.-V. (2009). Antibacterial activity of the leaf essential oil of *Blumea mollis* (D.Don) Merr. *World Journal Microbiol* *Niotechnol*, 1297-1300.
- Witayapan Nantitanon, S. c. (2007). Antioxidant and antimicrobial activities of *Hyptis suaveolens* essential oil. *Scientia Pharmaceutica*, 35-46.
- Halliwell, B. and Gutteridge, J.-M.-C.1999. "Free Radicals in Biology and Medicine". 3rd edition, Oxford University Press, Oxford, U.K.
- Finkel, T. and Holbrook, N.-J. 2000. Oxidants, oxidative stress and the biology of aging. *Nature*, 408: 239-247.
- Williams HM (2003). *Nutrition for health fitness and Sport*. 5th edition, published by Edward E. Bartell. U.S.A., pp. 209-210.
- Garrow JS (1993). *Human nutrition and dietetics*, 9th edition, publisher Churchill Livingstone UK, pp. 225-227.
- Gordon MN (1990). The mechanism of antioxidant action in vitro: Food antioxidant, Elsevier Appl. Sci., NY, pp. 1-18.
- Frankel EN (1991). Recent advances in lipid oxidation. *J. Sci. Fd. Agric.*, 54: 495-496.
- Shahidi F, Wanasundara PKJ (1992). Phenolic antioxidants, *Crit. Rev. Food Sci. Nutr.*, 32: 67-103.
- V. L. Singleton and Joseph A. Rossi Jr. (1965) Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents, *Am. J. Enol. Vitic* 1965 vol. 16 no. 3 144-158.
- Weisenthal, L.M. & Lippman, M.E. (1985). Clonogenic and non-clonogenic in vitro chemosensitivity assays. *Cancer Treat. Rep.*, 69, 615.
- Bei W, Peng W, Ma Y, Xu A (2005). Flavonoids from the leaves of *Diospyros kaki* reduce hydrogen peroxide-induced injury of NG108-15 cells. *Life Sci.*, 76(17): 1975-1988
- Zhao B (2005). Natural antioxidants for neurodegenerative diseases. *Mol Neurobiol.*, 31(1-3): 283-294.

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